

**REMARKS**

This is a full and timely response to the Office Action mailed January 27, 2005.

No claims have been amended in this response. Thus, claims 1-3 and 7-9 are currently pending for the Examiner's consideration, with claims 7-9 being withdrawn.

In view of this response, Applicant believes that all pending claims are in condition for allowance. Reexamination and reconsideration in light of the above claims and the following remarks is respectfully requested.

**Rejections under 35 U.S.C. § 112**

Claims 1-3 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Further, claims 1-3 are rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to enable one skilled in the art to practice the claimed invention. Applicant respectfully traverses these rejections.

In the Office Action, the Examiner argues that the experimental results in the specification do not show that the present invention “*accelerated the differentiation of undifferentiated mesenchymal cells to chondrocyte cells*”. Instead, the Examiner argues that Applicant’s data only shows that chondrocytes irradiated with ultrasound increased aggrecan protein production. In other words, the Examiner believes that the experimental results provide no evidence or teachings on the rate at which the original undifferentiated mesenchymal cells differentiated into chondrocytes. Thus, the Examiner has concluded that the disclosure and Examples do not show “*possession of*” and “*how to practice*” the claimed method of accelerating the differentiation of undifferentiated mesenchymal cells to chondrocyte cells. However, Applicant strongly disagrees with the Examiner in this regard.

In the Examples, Applicant showed that the amount of aggrecan in the undifferentiated mesenchymal stem cells of Group 3 (TGF- $\beta$  3(+)/ultrasound (+)) are increased about 1.85 fold higher than that of Group 2 (TGF-  $\beta$  3(+)) by the treatment of ultrasound after only ten days. It is important for the Examiner to understand that it usually takes about three weeks (21 days) for undifferentiated mesenchymal stem cells to differentiate to chondrocytes. Thus, the 1.85 fold increase in the amount of aggrecan in the undifferentiated mesenchymal stem cells of Group 3 over

that of Group 2 (TGF- $\beta$  3(+)) after treatment of ultrasound for only ten days is clearly due to the acceleration of differentiation of undifferentiated mesenchymal cells to chondrocyte cells and not due to the increased aggrecan protein production of differentiated chondrocytes. Thus, ultrasound treatment is clearly effective for increasing the differentiation of mesenchymal cells to chondrocytes by shortening the time of such differentiation.

In further support, histological examination (see Fig. 4) reveals that pellets treated with both TGF- $\beta$  3 and ultrasound (Group 3) are strongly stained with alcian blue as well as pellets treated with only TGF- $\beta$  3 (Group 2). Especially, the bottom half of Group 3 is better stained than only TGF- $\beta$  3 treated pellets (Group 2). This data shows that the extracellular matrix of cartilage, and chondroitin sulfate in the immature chondrocytes are increased higher than that of Group 2 (TGF- $\beta$  3(+)) by the treatment of ultrasound for ten days. Immunostaining pattern for aggrecan (see Fig. 5) shows similar result to that of alcian blue. Thus, these results further demonstrate that TGF- $\beta$  3 induces chondrocyte differentiation of mesenchymal stem cells in the pellets, and that ultrasound treatment enhances such differentiation.

Thus, in view of the above, withdrawal of these rejections is respectfully requested.

### **Rejections under 35 U.S.C. § 103**

Claims 1 and 2 remain rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Pittenger et al. (WO 98/32333) in view of Parvizi et al. Applicant respectfully traverses this rejection.

In maintaining the prior art rejections, the Examiner believes that since Applicant correlates the acceleration of differentiation of undifferentiated mesenchymal cells to chondrocyte cells by the production of aggrecan protein, once aggrecan is produced, the application of ultrasound in the present invention reads on the prior art references. The Examiner also believes that one skilled in the art would be motivated to apply ultrasound directly to a culture of undifferentiated mesenchymal cell to immediately expose newly differentiated chondrocytes to ultrasound. However, Applicant strongly disagrees with the Examiner's arguments in this regard.

As previously stated, to establish a *prima facie* case of obviousness, the prior art references must either alone or in combination teach the invention as a whole, including all the

limitations of the claims. Here, in this case, even if the Applicant accepts for the purpose of argument the Examiner's reasons for maintaining the rejection, the cited reference, in combination, still fails to teach or suggest the limitation "irradiating said undifferentiated mesenchymal cells with ultrasound to accelerate the differentiation of said undifferentiated mesenchymal cells to chondrocyte cells" (emphasis added).

This is due to the fact that the Examiner's argument does not address how the cited references teach or suggest "irradiating said undifferentiated mesenchymal cells with ultrasound to accelerate the differentiation of said undifferentiated mesenchymal cells to chondrocyte cells". Pittenger et al. teaches manufacturing artificial cartilage, comprising culturing undifferentiated mesenchymal stem cells in a chondroinductive medium that contains TGF- $\beta$ 3. Parvizi et al. teach irradiating a culture of chondrocyte cells with ultrasound to increase aggrecan m-RNA and protein by chondrocytes. More particularly, Parvizi et al. teach only that irradiating mature chondrocytes with ultrasound treatment increases aggrecan m-RNA and protein. The Examiner's argument that once aggrecan is produced, the application of ultrasound in the present invention reads on the prior art references, is irrelevant since the prior art references do not teach irradiating undifferentiated mesenchymal cells with ultrasound. Further, the present claims are only directed to the acceleration of differentiation of undifferentiated mesenchymal cells to chondrocyte cells with ultrasound. As a result, it expressly excludes the application of ultrasound for the purposes of increasing aggrecan production. Hence, it is clear that both Pittenger et al. and Parvizi et al. fail to teach or suggest irradiating undifferentiated mesenchymal cells with ultrasound.

With regard to the Examiner's argument that one skilled in the art would be motivated to apply ultrasound directly to a culture of undifferentiated mesenchymal cell to immediately expose newly differentiated chondrocytes to ultrasound, the Examiner has not cited a location in the cited references or in the prior art which would support such a motivation. As the Examiner already knows, the motivation or suggestion to combine or modify references in an obviousness rejection must be found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

A prior art reference that "teaches away" from the claimed invention is a significant factor to be considered in determining obviousness. In other words, it is improper to combine references where the references teach away from their combination. *In re Grasselli*, 713 F.2d 731, 743, 218 USPQ 769, 779 (Fed. Cir. 1983). Here, in this case, Parvizi et al. teach away from irradiating undifferentiated mesenchymal stem cells with ultrasound since Parvizi et al. teach only irradiating a culture of chondrocyte cells. Thus, one skilled in the art, based on the teachings of Parvizi et al., would not be motivated to irradiate undifferentiated mesenchymal stem cells with ultrasound since such teaching is not found in any of the cited references.

The Examiner countered such arguments in the Office Action but arguing that Parvizi et al. does not teach that ultrasound cannot be applied to undifferentiated mesenchymal cells or that adverse effects occur with the application of ultrasound. However, such arguments do not address how Parvizi et al. teach irradiating undifferentiated mesenchymal cells with ultrasound. Further, the fact that Parvizi et al. does not teach that ultrasound cannot be applied to undifferentiated mesenchymal cells or that adverse effects occur with the application of ultrasound does not constitute sufficient motivation to support an obviousness rejection. Under U.S. case law, the mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

It should also be noted that a *prima facie* case of obviousness based on similarity of methods is rebuttable by proof that the claimed method possess unexpectedly advantageous or superior properties. *In re Papesch*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963). In the Examples of the present specification, the Applicants have shown that that ultrasound treatment accelerates the differentiation of undifferentiated mesenchymal cells to chondrocyte cells. Such a result is clearly unexpected and superior to that which is taught in the cited references.

Thus, for these reasons, this rejection cannot be sustained and should be withdrawn.

Claims 1-3 remain rejected under 35 U.S.C. §103(a) as allegedly being obvious over Pittenger et al. (WO 98/32333) in view of Nishikori et al. Applicant respectfully traverses this rejection.

As stated above, Pittenger et al. teaches manufacturing artificial cartilage, comprising culturing undifferentiated mesenchymal stem cells in a chondroinductive medium that contains TGF- $\beta$  3. However, it fails to teach or suggest irradiating undifferentiated mesenchymal cells with ultrasound. Like Parvizi et al., such a deficiency is not cured by the teachings of Nishikori et al. since it also teaches irradiating *cultures of chondrocyte cells* with ultrasound. Specifically, Nishikori et al. teach irradiating *cultures of mature chondrocyte cells* with ultrasound to increase chondroitin sulfate synthesis by the chondrocytes which accelerate the production of complete cartilage from chondrocytes. Thus, the combination of Pittenger et al. (WO 98/32333) and Nishikori et al. cannot render obvious the present claims since they, in combination, fail to teach or suggest “irradiating said undifferentiated mesenchymal cells with ultrasound to accelerate the differentiation of said undifferentiated mesenchymal cells to chondrocyte cells” (emphasis added).

Further, for the same reasons as discussed above, one skilled in the art, based on the teachings and suggestions of Nishikori et al., would not be motivated to irradiate undifferentiated mesenchymal stem cells with ultrasound since such teaching, suggestion or motivation is not found in both Pittenger et al. and Nishikori et al.

Lastly, to further demonstrate the unexpected superior results of the present invention, Nishikori et al. teaches that the chondroitin sulfate content in ultrasound-treated group are twice as high as those in the control group, after 3 weeks of culture of chondrocytes (see Table I of Nishikori et al.). The experimental data of the present specification shows that ultrasound treatment accelerates the chondrocyte differentiation of mesenchymal stem cells after a culture of only 10 days.

Thus, withdrawal of the present rejection is respectfully requested.


**CONCLUSION**

For the foregoing reasons, all the claims now pending in the present application are believed to be clearly patentable over the outstanding rejections. Accordingly, favorable reconsideration of the claims in light of the above remarks is courteously solicited. If the Examiner has any comments or suggestions that could place this application in even better form, the Examiner is requested to telephone the undersigned attorney at the below-listed number.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 18-0013, under Order No. TEI-0128 from which the undersigned is authorized to draw.

Dated: March 24, 2005

Respectfully submitted,

By   
Lee Cheng  
Registration No.: 40,949

RADER, FISHMAN & GRAUER PLLC  
1233 20th Street, N.W. Suite 501 Washington,  
DC 20036  
(202) 955-3750  
Attorneys for Applicant

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